REMARKS

Applicants have further amended the claims in a further effort to clarify the claimed invention and to distinguish over the prior art. All of the claims have been canceled from the application and replaced with a new set of claims. Claim 54 is the main claim now in the application. The claims have been modified by the incorporation of several additional limitations in order to reduce the number of issues and concentrate upon the key aspects of the present invention. In particular, new claim 54 is now limited by the combination of original claims 1, 5, 8, 25, 27, 31 and 34 and by the restriction to TCII specific antibodies or antibody fragments as specific binding ligand. Claim 1 of the previous claim set relating to holo-TCII specific ligands has been deleted, as have the dependent claims now rendered unnecessary.

An additional restriction that the assay be capable of detecting holo-TCII at a concentration as low as 35 pM has also been included in claim 54. This is based on lines 2-5 of page 6 of Applicants' specification wherein it is disclosed as the lower range of normal cobalamin levels. Obviously, the test should be able to detect down to this level if the assay is to determine the possible presence of cobalamin deficiency.

It is essential in support of the amended claim set that the Examiner understand the species involved and the way in which the claimed assay functions. There are a number pf passages in the last office action where the Examiner believes that Applicants have argued two species to be "equivalent". This is not the case. Applicants have simply pointed out that in some cases measuring of one component (e.g. cobalamin at the end of the assay) is equivalent to measuring of another component (e.g. holo-TCII at the start of the assay) because of the way in which the sample is prepared using the specific binding ligands. Applicants would therefore like to make the following, purely factual, observations by way of background to the invention:

i. Three separate molecules are involved in the present application and these combine into four principal species. Cobalamin, otherwise known as vitamin B_{12} is a small organic macrocycle containing a cobalt atom at the centre, transcobalamin II (TCII) is a protein which binds to cobalamin, as is haptocorrin (HC). When the protein components are not bound to cobalamin, they are described as "apo-" and when cobalamin is bound, they are described as "holo-" thus, "TCII" includes holo-TCII (the complex of the TCII protein with B_{12}) and apo-TCII (the TCII protein in the absence of any bound ligand). In the body, almost no free B_{12} exists in solution but is present in the blood as a protein complex with HC or TCII. Of the four fractions (apo-HC, apo-TCII, holo-HC and holo-TCII), most of the protein content is HC and most of the cobalamin is present as holo-HC. Holo-TC is thus the least abundant of the four species in the sample and therefore the hardest to analyse.

ii. The level of holo-TCII in a patient with border-line cobalamin deficiency is thought to be around 35 pM. The minimum concentration of cobalamin that can be detected by known cobalamin immunoassays is theoretically about 40 pM but in practice around 90 pM.

iii. Clearly an assay that can only detect cobalamin at 90 pM or greater cannot be used to determine cobalamin deficiency by measuring the cobalamin component of holo-TCII at around 35 pM since this contains 35pM of cobalamin and is thus below the limit of detection.

iv. If the amount (i.e. number of moles) of a species (such a cobalamin) in a sample remains the same **but the volume of the sample is reduced** then the concentration must increase correspondingly. This is a mathematical certainty because concentration is defined as the amount of substance per unit volume.

v. Holo-TCII is a 1:1 complex of cobalamin with the TCII protein. Therefore, in a sample having holo-TCII as the only cobalamin containing species, the concentration of holo-TCII is the same as the concentration of cobalamin.

The key advantage of the present invention is that a specific binding ligand (antibody or fragment) is used to create a ligand bound fraction in which holo-TCII is the only species which contains cobalamin. The amount of cobalamin bound by the ligand can be determined by a simple experiment but will be at least 80% of the amount present as holo-TCII in the original sample. The cobalamin in this ligand bound fraction is then released, but rather than releasing it into the same volume of liquid as the original sample, a smaller volume of release solution is used so that the concentration of cobalamin is at least 3-fold greater than the original holo-TCII concentration but the ratio of these two concentrations is known. This is exemplified on the first two paragraphs of page 8 of Applicants' specification. In order for this step to function effectively, the specific binder must be very specific, must not leave much holo-TCII in the non-bound fraction (or this will be wasted) and must bind very little holo-HC (or an inaccurate result will be given). These features are now all explicitly recited in new claim 54 and are fully supported by Applicants' specification.

As can be seen from the above discussion, it is the combination of a ligand with high specificity and affinity, so as to avoid loss of holo-TCII or contamination with holo-HC, combined with release into a reduced volume that provides a combined separation and concentration step. Applicants most respectfully submit that all of the claims now present in the application are in full compliance with 35 USC 112 and are clearly patentable over the references of record.

Applicants have carefully considered each of the rejections in the Final Rejection and these rejections are specifically traversed in view of the further amendments to the claims and the level of one of ordinary skill in the art to which the invention pertains.

Applicants most respectfully submit that teachings of the specification must be considered in light of the level of one of ordinary skill in the art and that routine experimentation is permissible. Each of these rejections will now be considered with respect to the paragraph numbering in the Office Action.

4. The Official Action rejects the identified claims on the basis of addition of subject matter to the application. This rejection is specifically traversed. Applicants most respectfully submit that all of the wording used in the previous main claim related to subject matter which the skilled worker would consider clearly disclosed in the application as filed. Nonetheless, the wording was introduced in the hope of clarifying the claim in a manner acceptable to the Examiner. If the Examiner considers the claims both insufficiently specific and to add matter then this benefit is not achieved. As a result, new claim 54 as herewith submitted has been rewritten starting from the claim set as originally filed and incorporating the restrictions set out in original claims 1, 5, 8, 25, 27, 31 and 34 and by the restriction to TCII specific antibodies or antibody fragments as specific binding ligands. The wording now used is quite specific and clearly based on the specification and original claims as filed. Accordingly, it is most respectfully requested that this rejection be withdrawn.

5. The Official Action rejects to the coverage of specific binding ligands other than antibodies and antibody fragments on the grounds of lack of enablement. As indicated in previous responses, such ligands are considered fully enabled to one of ordinary skill in the relevant art without undue experimentation. However, in the interest of making progress with the present application, the main claim has been restricted to specify antibodies and fragments thereof. Accordingly, it is most respectfully requested that this rejection be withdrawn.

6. The Official Action urges that the steps relating the holo-TCII concentration in the original sample to the measured value are insufficiently specified. Applicants most respectfully submit that this correlation is central and fundamental to the functioning of the assay and it is essential that this be understood. It is most

important to appreciate that **nowhere in the claim is it suggested that the amount of cobalamin is increased**. It is only the concentration of cobalamin (that is to say the amount per unit volume) that is increased. This is achieved **not by increasing the amount of cobalamin but by reducing the volume in which this same amount is dissolved**. The Examiner's reference to not knowing "where the cobalamin is coming from" clearly suggests that this point has not been fully appreciated and it is now believed that it is clear as would be appreciated by one of ordinary skill in the art ot which the invention pertains.

Applicants wish to note that the assay functions by taking a sample which contains a certain amount of body fluid (say 100 $x10^{-6}$ litres) having a certain concentration of holo-TCII (this amount to be determined - say $Y \times 10^{-12}$ moles per litre). The total amount of holo-TCII in the sample is thus $100 \times 10^{-6} \times Y \times 10^{-12} = Yx10^{-16}$ moles. This contains $Yx10^{-16}$ moles of TCII protein and $Yx10^{-16}$ moles of cobalamin. Further cobalamin is present in the sample in the form of holo-HC and further TCII protein is present in the form of apo-TCII. The specific binding ligand binds the TCII components and ideally will bind all $Yx10^{-16}$ moles of holo-TCII and all of the apo-TCII but no HC. This is the "ligand bound fraction". The ligand binds none of the holo-HC, therefore the ligand bound fraction contains all of the TCII protein from the sample and $Yx10^{-16}$ moles of cobalamin, in the form of holo-TCII. The cobalamin from the ligand bound fraction is then released. However, this is "so affected that the concentration of the released cobalamin is at least 3 times greater than the concentration of holo-TCII in the initial sample". To do this, we simply need to release the cobalamin into a smaller volume - say 20 µl. The concentration of the release cobalamin will be $Yx10^{-16}$ moles in 20×10^{-6} litres or $5Y \times 10^{-12}$ moles per litre. In other words, the concentration of cobalamin released is known to be exactly 5 times as great as the holo-TCII concentration in the original sample. Although the amount of cobalamin has remained unchanged. Thus, not only has the process of the assay separated out the holo-TC from the holo-HC but at the same time, the value to be measured has been increased 5-fold. This places the

concentration of cobalamin from the holo-TCII in a patient with borderline cobalamin deficiency within the range of sensitivity of cobalamin assays. By this process, holo-TCII concentration may be used to assess cobalamin deficiency where, without this method, the sensitivity of assays having suitable throughput was insufficient.

In view of the above explanation, it is clear that the specific binding and concentration steps directly relate the holo-TCII concentration of the cell free sample to the cobalamin concentration released and measured in the final step. Where any of the binding or release steps are imperfect, the appropriate ratios will be trivially determinable by the skilled worker by the use of standard samples. Accordingly, it is most respectfully requested that this rejection be withdrawn.

7. The continued rejection of the claims as anticipated by Herbert has been carefully considered but is most respectfully traversed. As pointed out in previous responses, Applicant again note that there is no step described in Herbert which binds the TCII and releases the cobalamin contained in the holo-component thereof into a reduced volume to provide an at least 3-fold concentration increase. In addition, none of the further parameters now incorporated from the dependent claims is disclosed in Herbert. These are all claim limitations which cannot be ignored. Thus, the present claims are clearly novel over Herbert.

It is also pointed out for clarity that Applicants have never stated that "holo-TCII, cobalamin and TCII are all equivalent proteins" because, in fact, cobalamin is not a protein of any sort but a small organic molecule. Applicants have only ever pointed out the equivalence of measuring the concentrations of holo-TCII, cobalamin or the TCII protein, under the very specific circumstances of the present assay method (i.e. when all apo-TCII, and holo-HC have been removed). Accordingly, it is most respectfully requested that this rejection be withdrawn in view of the above comments and further amendments to the claims.

8. The obviousness rejection set forth in paragraph 8 has been carefully considered but is most respectfully traversed in view of the amendments to the claims

and the following comments. The term "analogue" is no longer present in the claims and thus the rejection of this section is obviated. It is also clear that the proteins described in Houts cannot be a "anologues" of the small organic molecule cobalamin since by their very nature they differ hugely in size and function as would be appreciated by one of ordinary skill in the art to which the invention pertains. Cobalamin is a coenzyme and thus exists to work with an enzyme to provide a function that the protein structure of the enezyme alone cannot. The proteins of Houts serve to bind cobalamin and are hundreds of times greater in molecular weight. An analogue must have a similar structure and function, which is not the case here.

In relation to the concentration step, the Examiner argues that only routine skill is required in "adjusting" the concentration of a component. This may be true where the concentration is adjusted by the addition of material. However, as described in detail above, **the amount of cobalamin in the sample cannot be adjusted in this way because this would destroy the value that is to be measured**. The concentration is increased by the recited specific binding and release steps so that the final cobalamin concentration is a known multiple of the original holo-TCII concentration. This step is neither obvious nor taught in any of the prior art. Accordingly, it is most respectfully requested that this rejection be withdrawn.

9. With respect to the rejection in paragraph 9, Applicants wish to note that the features considered by the Examiner not be indicated in the claims are now present, taken from the appropriate dependent claims and therefore this rejection should be withdrawn. A large number of very specific features are now explicitly recited in the main claim, claim 54, which are neither disclosed nor taught towards, either individually or in combination in any of the prior art documents. The necessary motivation and expectation of success are not found in the prior art. The amended claims thus cannot be considered obvious in light of the cited art. Accordingly, it is most respectfully requested that this rejection be withdrawn.

10. This rejection has been obviated by the cancellation of these claims from the application. Accordingly, it is most respectfully requested that this rejection be withdrawn.

11. The rejection in paragraph 11 has been carefully considered but is most respectfully traversed in view of the amendments to the claims. It is urged in the Official Action that it would be obvious to one of ordinary skill in the art to combine the antibodies of McLean with the assay method of Herbert and that Hoyle indicates the use of high affinity antibodies. Applicants do not believe this to be the case, but in any event, the method of Herbert does not provide a binding an release step giving a final cobalamin concentration of at least 3 times the original holo-TCII concentration. Thus, even if the Official Action is correct, which it is not, the combination of these references does not teach towards a method falling within the present claims. Accordingly, it is most respectfully requested that this rejection be withdrawn.

In view of the above comments and further amendments to the claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

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